WHAT IS CLAIMED IS:

1. An assay for determining the biochemical fitness of a biochemical target of a mutant replicating biological entity relative to its predecessor, comprising:

obtaining said predecessor,

determining the biochemical vitality of said biochemical target of said predecessor in the presence of a compound capable of inhibiting said biochemical target of said predecessor,

determining the biochemical vitality of said biochemical target of said mutant replicating biological entity in the presence of said compound, and

comparing the biochemical vitality of said biochemical target of said mutant replicating biological entity relative to the biochemical vitality of said biochemical target of said predecessor.

2. The assay of claim 1, wherein said predecessor is an infectious microorganism.

3. The assay of claim 2, wherein said infectious microorganism is a virus.

4. The assay of claim 3, wherein said virus is a retrovirus.

5. The assay of claim 4, wherein said retrovirus is HIV-1 or HIV-2.

6. The assay of claim 2, wherein said infectious microorganism is a malarial parasite.

7. The assay of claim 6, wherein said malarial parasite is a plasmodium species.

8. The assay of claim 2, wherein said infectious microorganism is a bacterium.

9. The assay of claim 1, wherein said predecessor is a cancer cell.

10. The assay of claim 9, wherein said cancer cell is a rapidly growing tumor cell.

Sub.aa1

~~11. The assay of any one of claims 1-10, wherein said biochemical target of said predecessor is an enzyme and said compound inhibits said enzyme of said predecessor.~~

~~12. The assay of any one of claims 3-5, wherein said biochemical target of said predecessor is a viral protease, a viral reverse transcriptase, a viral polymerase, a viral enzyme, or a viral protein.~~

~~13. The assay of claim 6 or 7, wherein said biochemical target of said malarial parasite is a plasmepsin, a plasmodial enzyme, or a protein.~~

14. The assay of any one of claims 1-10, wherein said biochemical target of said predecessor is an oligomer and said compound inhibits the oligomerization of said oligomer of said predecessor.

Sub a1

15. The assay of any one of claims 1-10, wherein said biochemical target of said predecessor is a protein and said compound inhibits a conformational change, ligand binding, or enzyme activity in said protein of said predecessor.

16. The assay of claim 11, wherein:

the biochemical vitality of the enzyme of said mutant replicating biological entity corresponds to $K_{inh-mut}$, $k_{cat-mut}$, and K_{M-mut}, and said biochemical vitality of the enzyme of said mutant replicating biological entity is defined by the relationship $K_{inh-mut}(k_{cat-mut}/K_{M-mut})$, and

the biochemical vitality of the enzyme of said predecessor corresponds to $K_{inh-pred}$, $k_{cat-pred}$, and K_{M-pred}, and said biochemical vitality of the enzyme of said predecessor is defined by the relationship $K_{inh-pred}(k_{cat-pred}/K_{M-pred})$,

wherein K_{inh} is an inhibition constant of said compound, k_{cat} is the biochemical catalytic rate, and K_M is the Michaelis constant.

17. The assay of claim 16, wherein $K_{inh-mut}$, $K_{inh-pred}$, $k_{cat-mut}$, $k_{cat-pred}$, K_{M-mut}, and K_{M-pred} are each measured.

18. The assay of claim 16 or 17, wherein K_{inh} is K_i.

Sub. a2

Sub a2

19. The assay of claim 16 or 17, wherein K_{inh} is K_d.

20. A method of administering a therapeutic compound that inhibits a biochemical target of a disease-causing replicating biological entity, comprising:

identifying at least one mutant capable of evolving from said disease-causing replicating biological entity,

determining a first biochemical vitality of said biochemical target of said disease-causing replicating biological entity in the presence of a first compound capable of inhibiting said biochemical target of said disease-causing replicating biological entity,

determining a first biochemical vitality of said biochemical target of said mutant replicating biological entity in the presence of said first compound,

determining a second biochemical vitality of said biochemical target of said disease-causing replicating biological entity in the presence of at least one additional compound capable of inhibiting said biochemical target of said disease-causing replicating biological entity,

determining a second biochemical vitality of said biochemical target of said mutant in the presence of said of at least one additional compound,

determining a first biochemical fitness of said biochemical target of said mutant relative to said disease-causing replicating biological entity by comparing the first biochemical vitality of said biochemical target of said mutant with the first biochemical vitality of said biochemical target of said disease-causing replicating biological entity,

determining a second biochemical fitness of said biochemical target of said mutant relative to said disease-causing replicating biological entity by comparing the second biochemical vitality of said biochemical target of said mutant with the second biochemical vitality of said biochemical target of said disease-causing replicating biological entity,

comparing the first biochemical fitness in the presence of said first compound with the second biochemical fitness in the presence of said at least one additional compound, and

administering, from among said first and said at least one additional compounds, a therapeutic compound which produces the lowest value for said first or said second biochemical fitness,

wherein said disease-causing replicating biological entity is less likely to develop resistance in the presence of said therapeutic compound.

21. The method of claim 20, wherein said replicating disease-causing replicating biological entity is an infectious microorganism.

22. The method of claim 21, wherein said infectious microorganism is a virus.

23. The method of claim 22, wherein said virus is a retrovirus.

24. The method of claim 23, wherein said retrovirus is HIV or HIV-2.

Sub.a13

25. The method of claim 21, wherein said infectious microorganism is a malarial parasite.

26. The method of claim 25, wherein said malarial parasite is a plasmodium species.

27. The method of claim 21, wherein said infectious microorganism is a bacterium.

Sub a9 4

28. The method of claim 20, wherein said replicating disease-causing replicating biological entity is a cancer cell.

29. The method of claim 28, wherein said cancer cell is a rapidly growing tumor cell.

30. The method of any one of claims 20-29, wherein said biochemical target of said disease-causing replicating biological entity is an enzyme and said compound inhibits said enzyme of said disease-causing replicating biological entity.

Sub a95

31. The method of any one of claims 22-24, wherein said biochemical target of said disease-causing replicating biological entity is a viral protease, a viral reverse transcriptase, a viral polymerase, a viral enzyme, or a viral protein.

32. The method of claim 25 or 26, wherein said biochemical target of said malarial parasite is a plasmepsin, a plasmodial enzyme, or a protein.

Sub. a9

33. The method of any one of claims 20-29, wherein said biochemical target of said disease-causing replicating biological entity is an oligomer and said compound inhibits the oligomerization of said oligomer of said disease-causing replicating biological entity.

34. The method of any one of claims 20-29, wherein said biochemical target of said disease-causing replicating biological entity is a protein and said compound inhibits a conformational change, ligand binding, or enzyme activity in said protein of said disease-causing replicating biological entity.

35. The method of claim 30, wherein:

the biochemical vitality of the enzyme of said mutant corresponds to $K_{inh\text{-}mut}$, $k_{cat\text{-}mut}$, and $K_{M\text{-}mut}$, and said biochemical vitality of the enzyme of said mutant is defined by the relationship $K_{inh\text{-}mut}(k_{cat\text{-}mut}/K_{M\text{-}mut})$, and

the biochemical vitality of the enzyme of said disease-causing replicating biological entity corresponds to $K_{inh\text{-}pred}$, $k_{cat\text{-}pred}$, and $K_{M\text{-}pred}$, and said biochemical vitality of the enzyme of said disease-causing replicating biological entity is defined by the relationship $K_{inh\text{-}pred}(k_{cat\text{-}pred}/K_{M\text{-}pred})$,

wherein K_{inh} is an inhibition constant of said compound, k_{cat} is the biochemical catalytic rate, and K_M is the Michaelis constant.

36. The method of claim 35, wherein $K_{inh-mut}$, $K_{inh-pred}$, $k_{cat-mut}$, $k_{cat-pred}$, K_{M-mut}, and K_{M-pred} are each measured.

37. The method of claim 35 or 36, wherein K_{inh} is K_i.

Sub a96

38. The method of claim 35 or 36, wherein K_{inh} is K_d.

39. The assay of claim 1, wherein said predecessor is a wild-type HIV strain and said mutant has at least one mutation in the biochemical target thereof.

40. The method of claim 20, wherein said disease-causing replicating biological entity is a wild-type HIV strain and said mutant has at least one mutation in the biochemical target thereof.

41. The assay of claim 1, wherein said predecessor has at least one mutation in the biochemical target thereof, and said mutant has at least two mutations in the biochemical target thereof.

42. The method of claim 20, wherein said disease-causing replicating biological entity has at least one mutation in the biochemical target thereof, and said mutant has at least two mutations in the biochemical target thereof.

43. The method of claim 39 or 40, wherein said mutant has at least one active site mutation.

Sub a97

AMENDED SHEET

44. The method of claim 41, wherein said predecessor or said mutant has at least one active site mutation.

45. The method of claim 42, wherein said disease-causing replicating biological entity or said mutant has at least one active site mutation.

46. A continuous fluorogenic assay for measuring the anti-HIV protease activity of a protease inhibitor, which method comprises:

adding a solution of HIV protease to at least a portion of a substrate stock solution, in which the substrate has the formula Ala-Arg-Val-Tyr-Phe(NO_2)-Glu-Ala-Nle-NH_2, to provide a substrate reaction solution;

measuring the fluorescence of said substrate reaction solution at specified time intervals;

adding said solution of HIV protease to an inhibitor-substrate solution comprising a protease inhibitor and said substrate stock solution, to provide an inhibitor-substrate reaction solution;

measuring the fluorescence of said inhibitor-substrate reaction solution at specified time intervals; and

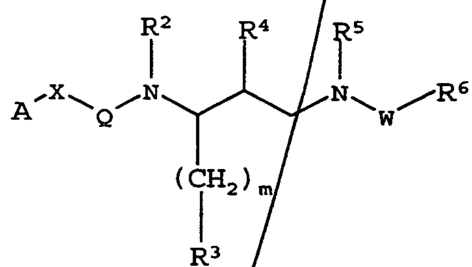
calculating the initial velocity of said inhibitor-substrate reaction solution by applying the equation: $V=V_0/2E_t(\{[K_i(1+S/K_m)+I_t-E_t]^2+4K_i(1+S/K_m)E_t\}^{1/2}-[K_i((1+S/K_m)+I_t-E_t])$, wherein V is the initial velocity of said inhibitor reaction solution, V_0 is the initial velocity of said substrate reaction solution, K_m is the Michaelis-Menten constant, S is the concentration of said substrate, E_t is

the concentration of said protease, and I_t is the concentration of said inhibitor, wherein the initial velocities indicates the anti-HIV protease activity of said protease inhibitor.

47. A method of preventing the development of drug resistance in an HIV-infected mammal, said method comprising administering to said HIV-infected mammal a drug resistance-inhibiting effective amount of a compound of the formula:

~~Sub. a98~~

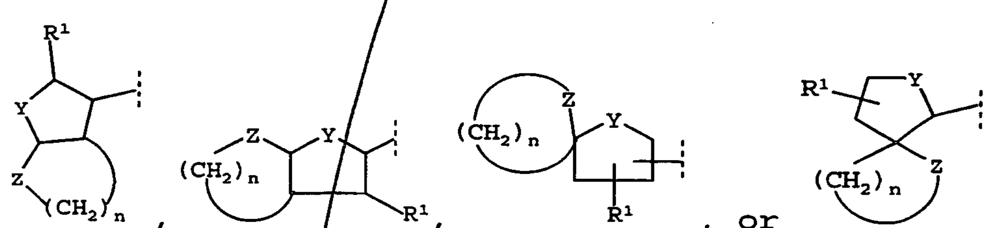
Sub. a1



(I),

or a pharmaceutically acceptable salt, a prodrug, or an ester thereof, or a pharmaceutically acceptable composition of said compound, said salt, said prodrug, or said ester thereof, wherein:

A is a group of the formula:



R^1 is H or an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a cycloalkylalkyl, an aryl, an aralkyl, a heterocycloalkyl, a heterocycloalkylalkyl, a heteroaryl, or a heteroaralkyl in which at least one hydrogen atom is optionally substituted with a substituent selected from the group consisting of OR^7, SR^7, CN, NO_2, N_3, and a

halogen, wherein R^7 is H, an unsubstituted alkyl, an unsubstituted alkenyl, or an unsubstituted alkynyl;

Sub.a1

Y and Z are the same or different and are independently selected from the group consisting of CH_2, O, S, SO, SO_2, NR^8, $R^8C(O)N$, $R^8C(S)N$, $R^8OC(O)N$, $R^8OC(S)N$, $R^8SC(O)N$, $R^8R^9NC(O)N$, and $R^8R^9NC(S)N$, wherein R^8 and R^9 are each selected from the group consisting of H, an unsubstituted alkyl, an unsubstituted alkenyl, and an unsubstituted alkynyl;

n is an integer from 1 to 5;

X is a covalent bond, CHR^{10}, $CHR^{10}CH_2$, CH_2CHR^{10}, O, NR^{10}, or S, wherein R^{10} is H, an unsubstituted alkyl, an unsubstituted alkenyl, or an unsubstituted alkynyl;

Q is C(O), C(S), or SO_2;

R^2 is H, a C_1-C_6 alkyl, a C_2-C_6 alkenyl, or a C_2-C_6 alkynyl;

m is an integer from 0 to 6;

R^3 is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl in which at least one hydrogen atom is optionally substituted with a substituent selected from the group consisting of alkyl, $(CH_2)_pR^{11}$, OR^{12}, SR^{12}, CN, N_3, NO_2, $NR^{12}R^{13}$, $C(O)R^{12}$, $C(S)R^{12}$, CO_2R^{12}, $C(O)SR^{12}$, $C(O)NR^{12}R^{13}$, $C(S)NR^{12}R^{13}$, $NR^{12}C(O)R^{13}$, $NR^{12}C(S)R^{13}$, $NR^{12}CO_2R^{13}$, $NR^{12}C(O)SR^{13}$, and a halogen, wherein:

p is an integer from 0 to 5;

R^{11} is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl in which at least one hydrogen atom is optionally substituted with a substituent selected from the group consisting of a halogen, OH, OCH_3, NH_2, NO_2, SH, and CN; and

R^{12} and R^{13} are independently selected from the group consisting of H, an unsubstituted alkyl, an unsubstituted alkenyl, and an unsubstituted alkynyl;

R^4 is OH, =O (keto), NH_2, or $NHCH_3$;

R^5 is H, a C_1-C_6 alkyl radical, a C_2-C_6 alkenyl radical, or $(CH_2)_qR^{14}$, wherein q is an integer form 0 to 5, and R^{14} is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl radical in which at least one hydrogen atom is optionally substituted with a substituent selected from the group consisting of a halogen, OH, OCH_3, NH_2, NO_2, SH, and CN;

Sub.a1

W is C(O), C(S), or SO_2; and

R^6 is a cycloalkyl, heterocycloalkyl, aryl, or heteroaryl radical in which at least one hydrogen atom is optionally substituted with a substituent selected from the group consisting of a halogen, OR^{15}, SR^{15}, $S(O)R^{15}$, SO_2R^{15}, $SO_2NR^{15}R^{16}$, $SO_2N(OH)R^{15}$, CN, CR^{15}=NR^{16}, CR^{15}=$N(OR^{16})$, N_3, NO_2, $NR^{15}R^{16}$, $N(OH)R^{15}$, $C(O)R^{15}$, $C(S)R^{15}$, CO_2R^{15}, $C(O)SR^{15}$, $C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$, $C(O)N(OH)R^{15}$, $C(S)N(OH)R^{15}$, $NR^{15}C(O)R^{16}$, $NR^{15}C(S)R^{16}$, $N(OH)C(O)R^{15}$, $N(OH)C(S)R^{15}$, $NR^{15}CO_2R^{16}$, $N(OH)CO_2R^{15}$, $NR^{15}C(O)SR^{16}$, $NR^{15}C(O)NR^{16}R^{17}$, $NR^{15}C(S)NR^{16}R^{17}$, $N(OH)C(O)NR^{15}R^{16}$, $N(OH)C(S)NR^{15}R^{16}$, $NR^{15}C(O)N(OH)R^{16}$, $NR^{15}C(S)N(OH)R^{16}$, $NR^{15}SO_2R^{16}$, $NHSO_2NR^{15}R^{16}$, $NR^{15}SO_2NHR^{16}$, $P(O)(OR^{15})(OR^{16})$, an alkyl, an alkoxy, an alkylthio, an alkylamino, a cycloalkyl, a cycloalkylalkyl, a heterocycloalkyl, a heterocycloalkylalkyl, an aryl, an aryloxy, an arylamino, an arylthio, an aralkyl, an aryloxyalkyl, an arylaminoalkyl, an aralkoxy, an (aryloxy)alkoxy, an (arylamino)alkoxy, an (arylthio)alkoxy, an aralkylamino, an (aryloxy)alkylamino, an (arylamino)alkylamino, an

(arylthio)alkylamino, an aralkylthio, an (aryloxy)alkylthio, an (arylamino)alkylthio, an (arylthio)alkylthio, a heteroaryl, a heteroaryloxy, a heteroarylamino, a heteroarylthio, a heteroaralkyl, a heteroaralkoxy, a heteroaralkylamino, and a heteroaralkylthio,

Sub a1

wherein R^{15}, R^{16}, and R^{17} are H, an unsubstituted alkyl, or an unsubstituted alkenyl,

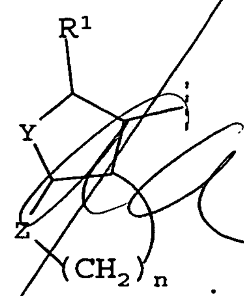
wherein, when at least one hydrogen atom of R^6 is substituted with a substituent other than a halogen, OR^{15}, SR^{15}, CN, N_3, NO_2, $NR^{15}R^{16}$, $C(O)R^{15}$, $C(S)R^{15}$, CO_2R^{15}, $C(O)SR^{15}$, $C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$, $NR^{15}C(O)R^{16}$, $NR^{15}C(S)R^{16}$, $NR^{15}CO_2R^{16}$, $NR^{15}C(O)SR^{16}$, $NR^{15}C(O)NR^{16}R^{17}$, or $NR^{15}C(S)NR^{16}R^{17}$, at least one hydrogen atom on said substituent is optionally substituted with a halogen, OR^{15}, SR^{15}, CN, N_3, NO_2, $NR^{15}R^{16}$, $C(O)R^{15}$, $C(S)R^{15}$, CO_2R^{15}, $C(O)SR^{15}$, $C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$, $NR^{15}C(O)R^{15}$, $NR^{15}C(S)R^{16}$, $NR^{15}CO_2R^{16}$, $NR^{15}C(O)SR^{16}$, $NR^{15}C(O)NR^{16}R^{17}$, or $NR^{15}C(S)NR^{16}R^{17}$; or

R^5 and R^6 together with the N-W bond of formula (I) comprise a 12 to 18 membered ring, comprising at least one additional heteroatom in the ring skeleton other than the nitrogen of said N-W bond; and

wherein a mutant virus that is capable of evolving from the HIV virus infecting said mammal has lower fitness, relative to said HIV virus infecting said mammal, in the presence of said compound.

48. The method of claim 47, wherein A is a group of the formula:

Qa



Sub. aa9

49. The method of claim 47 or 48, wherein:

when R^1 is an alkyl, it is a C_1-C_6 alkyl;

when R^1 is an alkenyl it is a C_2-C_6 alkenyl;

when R^1 is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl, R^1 is a 4-7 membered ring;

when R^7, R^8 or R^9 is an unsubstituted alkyl, it is a C_1-C_6 unsubstituted alkyl;

when R^7, R^8 or R^9 is an unsubstituted alkenyl, it is a C_2-C_6 unsubstituted alkenyl;

R^3 is a 4-7 membered ring;

R^{11} is a 4-7 membered ring;

when R^{12} or R^{13} is an unsubstituted alkyl, it is a C_1-C_6 unsubstituted alkyl;

when R^{12} or R^{13} is an unsubstituted alkenyl, it is a C_2-C_6 unsubstituted alkyl;

when R^{14} is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl, R^{14} is a 4-7 membered ring;

when R^6 is a cycloalkyl, a heterocycloalkyl, aryl, or a heteroaryl, R^6 is a 4-7 membered ring;

when R^6 is substituted with a substituent that is an alkyl, an alkylthio, or an alkylamino, the substituent comprises from one to six carbon atoms; and

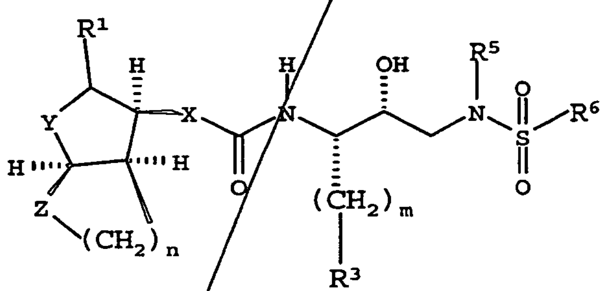
when R^6 is substituted with a substituent that is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl, the substituent is a 4-7 membered ring;

or a pharmaceutically acceptable salt, a prodrug, or an ester thereof.

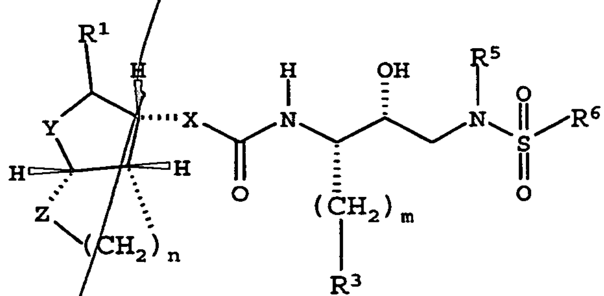
Sub. a29

50. The method of any one of claims 47-49, wherein Q is C(O), R^2 is H, and W is SO_2, or a pharmaceutically acceptable salt, a prodrug, or an ester thereof.

51. The method of claim 48, wherein said compound is represented by the formula:

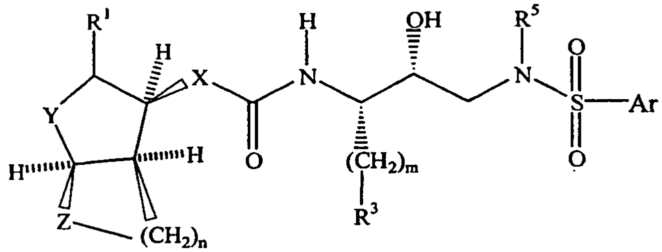


(IA) or

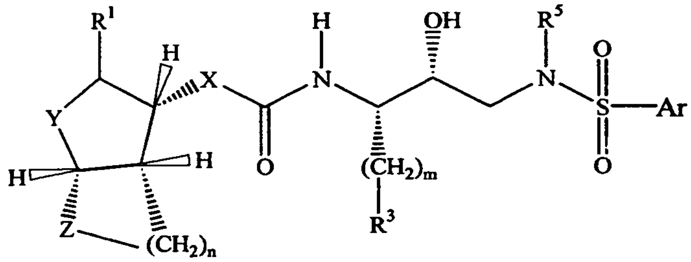


(IB).

52. The method of claim 51, wherein said compound is represented by the formula:



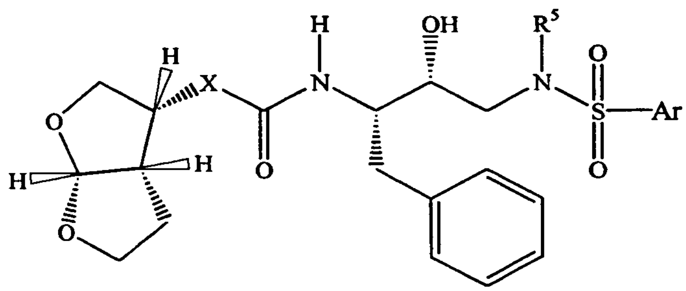
(IC) or



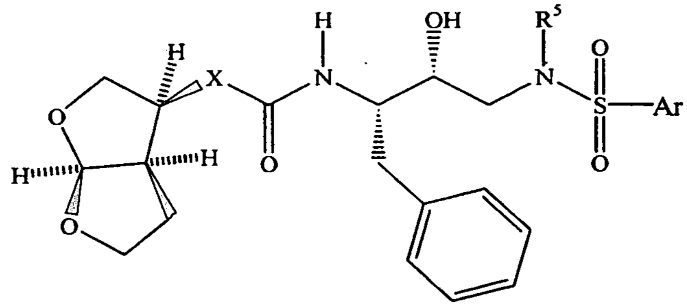
(ID),

wherein Ar is a phenyl which is optionally substituted with a substituent selected from the group consisting of methyl, amino, hydroxy, methoxy, methylthio, hydroxymethyl, aminomethyl, and methoxymethyl.

53. The method of claim 52, wherein said compound is represented by the formula:



(IE) or



(IF).

54. The method of claim 52 or 53, wherein X is oxygen.

55. The method of claim 52 or 53, wherein R^5 is isobutyl.

Sub. a10

56. The method of claim 52 or 53, wherein Ar is a phenyl substituted at the para-position.

57. The method of claim 52 or 53, wherein Ar is a phenyl substituted at the meta-position.

58. The method of claim 52 or 53, wherein Ar is a phenyl substituted at the ortho-position.

59. The method of claim 52 or 53, wherein Ar is selected from the group consisting of para-aminophenyl, para-toluyl, para-methoxyphenyl, meta-methoxyphenyl, and meta-hydroxymethylphenyl.

60. The method of any one of claims 47, 48, or 51-53, wherein said HIV-infected mammal is infected with a wild-type HIV.

61. The method of any one of claims 47, 48, or 51-53, wherein said HIV-infected mammal is infected by a mutant HIV with least one protease mutation.

Sub aa10

62. The method of any one of claims 47, 48, or 51-53, wherein said HIV-infected mammal is infected by a mutant HIV having at least one reverse transcriptase mutation.

add aa11

add a2